Biochemistry

IRON REMOVAL AND CRYSTALLIZATION OF HORSE SPLEEN FERRITIN FOR RADIATION DAMAGE STUDIES

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X-ray diffraction of protein crystals is a powerful technique used to determine the structure of proteins. However, the protein crystals undergo degradation due to the radiation they are exposed to during this technique. Crystals of protein with varying total absorption cross sections were grown for use in radiation damage studies. Horse spleen ferritin was the protein of choice. Commercially obtained ferritin is known to contain monomers and oligomers; the protein was purified to obtain a homogenous fraction of monomers suitable for crystallization. A portion of the iron in the ferritin iron-mineral core was removed by treatment with dihidroxyfumaric acid (DHF) and ethylenediaminetetraacetic acid (EDTA). This step was responsible for varying the molecular weight, and thus the total absorption cross-section of the protein. Two spectrophotometric methods were employed to determine the amount of iron removed. Modified protein was concentrated prior to crystallization by micro-centrifugation. The protein samples were crystallized by hanging drop vapor diffusion. Once grown, the crystals were used in subsequent radiation damage studies performed at the Cornell High Energy Synchrotron Source (CHESS).